REMARKS

Support for the new claims is found throughout the specification, including the claims, as originally filed. No new matter is introduced by the new claims. For example, Tables 5, 5A, and 5B show nine refolded pneumolysin polypeptides, modified in the region comprising amino acids 1 to 257, that have attenuated hemolytic activity. Pages 12-23 of the specification describe methods for producing and identifying modified pneumolysin. Pages 36-42 describe vaccines, antibodies, conjugate molecules, and pharmaceutical compositions. Thus, the newly presented claims are fully supported by the specification as originally filed.

RESPONSE TO § 112, SECOND PARAGRAPH, REJECTION

Claims 1-7, 22-26, and 31-34 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim subject matter. In particular, claims 1 and 2 were rejected for reciting "refoldable," claim 1 for reciting "similar," and claim 4 for reciting "the amino acid sequence Formula I SEQ ID NO:3." The new claims do not contain the above recitations. Applicants respectfully request withdrawal of this rejection.

RESPONSE TO § 112, FIRST PARAGRAPH, REJECTION

Claims 1-7, 22-26, and 31-34 were rejected under 35 U.S.C. § 112, first paragraph, for containing subjected matter not described in the specification in a way to reasonably convey to a skilled person that the inventors had possession of the claimed invention. In particular, the Examiner contends that the proviso "that the substitution is not solely a substitution of isoleucine for threonine at position 172" is new matter. Applicants respectfully disagree with this ground of rejection.

The proviso merely excludes a prior art polypeptide from the claims; a

polypeptide described in applicants' specification at pages 5 and 6. As the Examiner points out, the term refoldable also excludes the same prior art polypeptide, i.e., Lock's Ply Thr 172→Ile, from the claims. In the interest of moving forward with prosecution of this case, applicants do not include the proviso in the present claims. Applicants assert, however, that a pneumolysin polypeptide consisting solely of a Thr172→Ile substitution is not encompassed by the present claims which describe a refolded polypeptide. Applicants respectfully request withdrawal of this ground of rejection.

Claim 33 was rejected for reciting amino acid position "225." The present claims do not recite amino acid position "225." Therefore, applicants respectfully request withdrawal of this rejection.

Claims 1-7, 22-26 and 31-34 were rejected under 35 U.S.C. § 112, first paragraph, for containing subject matter not described in the specification in a way to enable a skilled person to make and use the invention. In particular, the Examiner contends that the proviso "that the substitution is not solely a substitution of isoleucine for threonine at position 172" means that the polypeptide should have at least a substitution of isoleucine for threonine at position 172 and at least one amino acid substitution in the region comprising amino acid residues 1 to 257. The Examiner's contention clearly misconstrues the claim. As described above, the proviso merely excludes a prior art polypeptide from the claims. The term "refolded" similarly excludes Ply Thr 172→Ile from the claims. However, the present claims do not recite the proviso and applicants respectfully request withdrawal of this rejection.

Claims 1-7, 22-26 and 31-34 were rejected under 35 U.S.C. § 112, first paragraph, because the specification does not reasonably provide enablement for a skilled person to make and use the invention commensurate in scope with the claim. The Examiner refers to several

prior art mutations, in a region outside the region of amino acids 1 to 257 described in applicants claims, to support the allegation that attenuation of hemolytic activity in pneumolysin by random mutation is an unpredictable event. Applicants respectfully disagree with this rejection.

Applicants' invention does not require a skilled person to predict, a priori, which amino acid substitution will produce an attenuated, refolded pneumolysin. Applicants' invention provides a method which reliably produces a refolded pneumolysin with attenuated hemolytic activity; a method that does not require undue experimentation. The Examiner has provided no evidence indicating that practicing applicants' invention would not produce an attenuated, refolded pneumolysin, nor that practicing applicants method, as opposed to prior art methods, would involve undue experimentation. The Examiner merely cites to several prior art methods and alleges that they are unpredictable. However, the Examiner has not provided any reason why the alleged unpredictability of the prior art methods has any relation to applicants' method.

The Examiner's reference to applicants' specification as acknowledging that several positions that fall in the range of amino acids 1-257 are not associated with decreases in hemolytic activity also misapplies the law as it relates to enablement. As a general rule, claims are not necessarily invalid even if they encompass some inoperative embodiments. *Atlas Powder Co. v. E.I. Du Pont De Nemours & Co.*, 750 F.2d 1569, 1576 (Fed. Cir. 1984)("'It is not a function of the claims to specifically exclude . . . possible inoperative substances . . . .'") (quoting *In re Dinh-Nguyen*, 492 F.2d 856, 858-59 (C.C.P.A. 1974)).

> [M]any patented claims read on vast numbers of inoperative embodiments in the trivial sense that they can and do omit 'factors which must be presumed to be within the level of ordinary skill in the art,' *In re Skrivan*, 427 F.2d 801, 806 (C.C.P.A. 1970), and therefore read on embodiments in which such factors *may be* included in such a manner as to make the embodiments inoperative. There is nothing wrong with this so long as it would be obvious to one of ordinary skill in the relevant art how to

include those factors in such manner as to make the embodiment operative rather than inoperative.

In *In re Angstadt*, 537 F.2d 498 (C.C.P.A. 1976), the court considered the subject matter of the application in issue, catalytic processes, unpredictable. The court also acknowledged that the scope of enablement varies inversely with the degree of unpredictability involved. The court even stated that "[a]ppellants have apparently not disclosed every catalyst which will work; they have apparently not disclosed every catalyst which will not work. *Id.* at 502. The court decided that "appellants are not required to disclose every species encompassed by their claims even in an unpredictable art such as the present record presents, each case must be determined on its own facts." *Id.* at 503. The present case, like the case in *Angstadt*, provides the public with a large but finite list of variables to choose from in preparing the object of the invention. Neither of the discovered processes, *Angstadt's* and present applicants', are complicated and no special equipment or reaction conditions are required. Thus, here, like in the case of *Angstadt*, there is no basis for concluding that persons skilled in this art, armed with the specification and its examples, would not easily be able to determine which modified pneumolysin polypeptides are refolded and have attenuated activity and which do not.

Like the applicants in *Angstadt*, the present applicants provide working examples of their invention. "If one skilled in this art wished to make and use a [refolded, attenuated pneumolysin] other than those disclosed, [they] would merely read [applicants'] specification for directions how to make [a refolded, attenuated pneumolysin,] and could then determine whether [refolded, attenuated pneumolysin is], in fact, formed." See, *In re Angstadt* at 503. Since applicants have provided a simple method for producing refolded, attenuated pneumolysin, the experimentation required to determine additional refolded, attenuated pneumolysin would not be undue and certainly would not require ingenuity beyond that to be expected of one of ordinary

skill in the art.

In addition, the court noted that "the PTO has the burden of giving reasons, supported by the record as a whole, why the specification is not enabling. Showing that the disclosure entails undue experimentation is part of the PTO's initial burden; this court has never held that evidence of the necessity for any experimentation, however slight, is sufficient to require the applicant to prove that the type and amount of experimentation needed is not undue. *** The key word is 'undue,' not 'experimentation.'" *In re Angstadt* at 504, citations omitted.

Regarding the Wands factors, applicants point out that they "are illustrative, not mandatory. What is relevant depends on the facts . . . ." *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd.*, 927 F2d 1200, 1213 (Fed Cir. 1991). The Examiner's analysis must consider all the evidence, and any conclusion of non-enablement must be based on the evidence as a whole. *In re Wands*, 858 F.2d 731, 737 and 740, emphasis added. The Examiner merely cites to several prior art methods of attenuating pneumolysin, and cites to several statements in applicants' specification that teach a skilled person how to practice the invention and avoid non-working embodiments. The Examiner fails to provide any evidence concerning applicants' method that would suggest undue experimentation would be required to practice applicants' invention.

The new claims require at least one amino acid substitution in the first 257 amino acids of the polypeptide. Polypeptides within the scope of the claims may be obtained by random mutation of a nucleic acid molecule encoding amino acids 1-257 of SEQ ID No: 3 and expressing the mutated nucleic acid in a host cell. The expressed protein is assayed for hemolytic activity, and those polypeptides which have attenuated hemolytic activity, substantially similar molecular weight as native wild-type pneumolysin, and are refoldable are identified and selected. Although the potential number of different polypeptides is large,

applicants provide nine working examples, some with single amino acid substitutions, some with multiple amino acid substitutions.

The state of the art and amount of direction provided by applicants' specification are such that one of ordinary skill in the art can practice applicants' claimed invention without undue experimentation. For example, of approximately 10,000 colonies, 400 were randomly selected for evaluation according to the following criteria: (1) low hemolytic activity, (2) substantially full-length, (3) partially soluble, and (4) monomeric and refoldable. Nine different polypeptides within the scope of applicants' claims were obtained; five with multiple mutations and four additional polypeptides with single mutations which were derived from the original five multiply mutated polypeptides.

The Examiner has not provided any evidence that a skilled person could not repeat applicants' process and obtain additional polypeptides that would fall within the scope of the amended claims. The Examiner relies on instances of an amino acid substitution either affecting the refoldability of a pneumolysin polypeptide or not effecting attenuation of hemolytic activity. However, the focus of analysis should not only be on the instances of mutations resulting in pneumolysin polypeptides not falling within the scope of applicants' claims. Indeed, not all mutations result in the production of pneumolysin polypeptide having desired characteristics; only 200 of 400 randomly mutated clones were selected based on a hemolytic assay. Rather, the focus of analysis should properly be concentrated on whether one skilled in the art, when practicing applicants' invention, will predictably create pneumolysin polypeptides that fall within the scope of the present claims. An analysis of this question in light of the Wands factors leads to the answer that, applicants' invention predictably produces the claimed pneumolysin polypeptides possessing the desired characteristics.

Applicants describe a method that does not depend on predicting whether a particular mutation or set of mutations will attenuate hemolytic activity and allow for protein folding. Rather applicants' method produces many mutants with attenuated hemolytic activity and proper folding without undue experimentation. Thus, the proper question is whether applicants' invention will predictably create pneumolysin polypeptides that fall within the present claims, and not whether pneumolysin polypeptides that do not fall within the present claims may also be created. The specification provides substantial direction as well as working examples for a skilled person to determine which pneumolysin polypeptides are refolded and attenuated and which are not.

Contrary to the Examiner's contentions, applicants have demonstrated the ability to obtain useful polypeptides with a sufficiently high level of probability. In fact, applicants have demonstrated that of 400 clones examined, more than 200 had attenuated activity (> 50%). Of these, 200 were chosen and examined for full-length expression. Fifty-eight, or 29% (14.5% overall), were found that expressed modified pneumolysin with about the same molecular weight as native pneumolysin. Of these fifty-eight, five clones (8.6% or 1.25% overall) were found to express refoldable pneumolysin polypeptide which was expressed in high yield, and four additional clones were derived from these for a total of 9 clones. Thus, it is clear that one skilled in the art can, predictably and reliably, obtain refolded pneumolysin with attenuated hemolytic activity according to applicants' invention.

Applicants' own evidence also demonstrates that obtaining a refoldable protein is a predictable event according to the invention. Fifty-eight clones which exhibited attenuated hemolytic activity and were of about full-length were examined for protein expression in the soluble fraction and for the ability of the expressed proteins to be denatured and refolded. Of the

clones that passed all these tests, five (8.6% or 1.25% overall) were chosen which exhibited very high levels of expression. The five clones (pNVJ1, pNVJ20, pNVJ22, pNVJ45 and pNVJ56) which exhibited attenuated hemolytic activity, full-length and refoldability all contained multiple mutations. See Example 5. Based on the amino acid substitutions of these five clones, four additional single mutation pneumolysin polypeptides (pNV103, pNV207, pNV111 and pNV211) were obtained by site directed mutagenesis. Thus, by following applicants' teachings, one of skill in the art would predict that other mutants within the scope of applicants' claims would be obtained, in addition to those described above, without undue experimentation. Thus, the amount of experimentation necessary to discover new and useful pneumolysin polypeptides is not undue since the process of random mutation and screening for hemolytic activity, protein size, and protein refoldability could be accomplished by routine and well-known methods.

The Examiner contends that applicants' discovery of a single mutation at position 243 that resulted in insoluble inclusion bodies is evidence that refoldability is an unpredictable event. However, when properly considered in an analysis similar to that of Wands, the exact opposite conclusion is reached. Of the fifty-eight clones that were of about full-length and exhibited attenuated hemolytic activity, five were found that were refoldable and expressed in high yields. Assuming that the remaining fifty-three clones were refoldable but not expressed in high yields (these are still within the scope of the claims), then 14.5% of the 400 clones originally examined fall within the scope of the claims. Even assuming that none of the remaining fifty-three clones were refoldable, the five found that were refoldable and expressed in high yields plus the 4 clones derived from them represents a 2.25% success rate. The Examiner has not provided any evidence that repeating applicants' procedure would not produce at least a 2.25% success rate. Even in Wands, appellants only had 2.8% probability of obtaining useful

clones. In addition, among the claims which were appealed in Wands and which the court found to be enabled, were those pertaining to chemically modified IgM antibodies immunoreactive to HbsAg. See, U.S. Patent No. 4,879,219 which issued from Wands et al. application 188,735. Thus, it is clear that the methods and compositions covered by applicants' claims are sufficiently reproducible to enable one skilled in the art to obtain other useful polypeptides. Therefore, reconsideration and withdrawal of the Section 112, first paragraph, rejection is respectfully requested.

AUTHORIZATION

No fee is deemed necessary in connection with the filing of this Communication. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 13-4500, Docket No. 1758-4036US2. A DUPLICATE COPY OF THIS COMMUNICATION IS ATTACHED.

Respectfully submitted,

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